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Evaluation of phytochemicals and mosquitocidal activity of *Calophyllum inophyllum* extract

Baskaran Blessy Evangelin Sheeba, Sabapathy Vijayakumar, Palanivel Vanishree, Shanmugasundram Somu, Murugaiyan Annathurai
Department of Zoology, A Veeriya Vandayar Memorial Sri Pushpam College, A Institution, Affiliated to Bharathidasan University, Poondi, Thanjavur, Tamil nadu, India

Abstract
The increasing resistance of Aedes aegypti mosquitoes to synthetic pesticides demands the investigation of sustainable and environmentally acceptable options for vector control. This research assessed the effectiveness of high-dose aqueous Calophyllum *inophyllum* leaf extract, rich in phytochemicals, against *Ae. aegypti* in laboratory settings. The extract was evaluated for its larvicidal, pupicidal, ovicidal, and adulticidal properties at different doses. Phytochemical analysis identified the existence of bioactive substances, including flavonoids, tannins, saponins, and alkaloids, recognised for their insecticidal characteristics. The results demonstrated significant mortality rates in larval and pupal stages, with dose-dependent effects observed. Ovicidal activity was evident through the inhibition of egg hatching, while adulticidal effects were marked by reduced survival and fecundity in treated mosquitoes. The study highlights the potential of *C. inophyllum* leaf extract as a sustainable and effective tool for integrated mosquito management, offering a promising alternative to chemical-based insecticides. Further research is recommended to optimize extraction methods, assess field efficacy, and evaluate environmental safety.

Keywords: *Calophyllum inophyllum*, *Aedes aegypti*, phytochemicals, larvicidal, pupicidal, ovicidal adulticidal sustainable vector control

Introduction

Mosquitoes are among the most significant vectors of human diseases, responsible for transmitting a wide range of pathogens that cause morbidity and mortality worldwide. The significance of mosquito management is paramount, since it is essential for preserving public health, maintaining ecosystems, and ensuring economic stability. Mosquitoes serve as vectors for several life-threatening infections, such as malaria, dengue fever, Zika virus, chikungunya, yellow fever, and West Nile virus. These illnesses jointly impact millions of individuals each year, especially in tropical and subtropical areas where mosquito populations flourish. In 2021, malaria was responsible for around 247 million cases and 619,000 fatalities, mostly in sub-Saharan Africa (WHO, 2022) [9]. Dengue fever, predominantly carried by Aedes aegypti mosquitoes, had a significant surge in occurrence, with over 5 million cases documented worldwide in 2019 (WHO, 2020) [10]. Effective mosquito control reduces the transmission of these diseases, preventing outbreaks and saving lives (Kovendan et al. 2024) [2].The *Aedes aegypti* mosquito serves as a principal vector for many arboviral infections, including as dengue fever, chikungunya, Zika virus, and yellow fever, which provide considerable public health issues worldwide (WHO, 2020) [11]. These illnesses account for millions of infections each year, especially in tropical and subtropical areas, where environmental circumstances promote the spread of *Ae. aegypti* (Guzman & Harris, 2015) [5]. Traditional control strategies rely heavily on synthetic insecticides, such as organophosphates and pyrethroids, to target mosquito populations. However, the overuse of these chemicals has led to the development of widespread insecticide resistance in *Ae. aegypti* populations, rendering many conventional control methods ineffective (Hemingway et al., 2016) [6]. Additionally, the environmental toxicity and non-target effects of synthetic insecticides have raised concerns about their sustainability and ecological impact (Devine et al., 2009) [4]. These challenges have spurred the search for alternative, eco-friendly, and sustainable approaches to mosquito control (Rajaganesh and Murugan, 2024). The use of aqueous extracts for mosquito control is particularly advantageous due to their simplicity of preparation, cost-effectiveness, and reduced environmental impact compared to organic solvent-based extracts (Benelli et al., 2019) [3]. High-dose aqueous extracts of *C. inophyllum* leaves have shown promise in preliminary studies, exhibiting potent larvicidal and adulticidal effects against mosquito vectors (Lakshmi et al., 2018) [7]. However, comprehensive evaluations of their efficacy across all life stages of *Ae. aegypti*—including eggs, larvae, pupae, and adults—are limited. Understanding the full spectrum of activity is crucial for developing integrated vector management strategies that target multiple stages of the mosquito life cycle, thereby enhancing the sustainability and effectiveness of control measures (Achee et al, 2019) [1]. This study aims to evaluate the phytochemical composition and insecticidal potential of high-dose aqueous *C. inophyllum* leaf extract against *Ae. aegypti* under laboratory conditions. Specifically, the extract will be tested for its larvicidal, pupicidal, ovicidal, and adulticidal activities, with the goal of identifying its potential as a sustainable and eco-friendly tool for mosquito control. By targeting all life stages of the mosquito, this approach seeks to disrupt the vector's life cycle and reduce population densities, thereby mitigating the transmission of mosquito-borne diseases. Furthermore, the study will explore the relationship between the phytochemical profile of the extract and its insecticidal efficacy, providing insights into the mechanisms underlying its activity.

Materials and Methods

Collection of *Calophyllum inophyllum* leaves

Fresh Calophyllum inophyllum leaves were procured from the Thanjavur area in Tamil Nadu, India. The leaves were recognised by a taxonomist from the Department of Botany at Bharathiar University, Coimbatore, Tamil Nadu, India.

Preparation of *Calophyllum inophyllum* leaves extract

The *C. inophyllum* leaves were rinsed with tap water and air-dried in the shade at ambient temperature (28±2°C) for a duration of 10 to 15 days. The desiccated *C. inophyllum* leaves were pulverised using a blender mixer grinder. Twenty grammes of leaf powder, encased in Whatman filter paper, were placed into a Soxhlet apparatus (Borosil Glass Works Ltd, Mumbai, India). The extraction was conducted using methanol (Loba Chemie Pvt. Ltd., Mumbai, India; 99% purity) at a concentration of 100%, over a duration of 72 hours, with the temperature maintained between 30-40°C. The yield extract was 100g, which was evaporated to dryness using a rotary vacuum evaporator. The resultant dried residues were kept in airtight bottles in a refrigerator for future use.

Qualitative Analysis

Preliminary Phytochemical analysis

The extract of *C. inophyllum* leaves underwent chemical analyses to identify several phytoconstituents, using the standard protocols outlined by Harborne (1984) and Trease and Evans (1979). The following procedures were used for the qualitative chemical analysis of several phytochemicals to provide a broad understanding of the phyto-constituents found in the extract of *C. inophyllum* leaves.

Gas Chromatography—Mass Spectroscopy analysis

The methanolic extract of *C. inophyllum* leaves was analysed using GC-MS (SHIMADZU QP2010) with an Elite-DB-5M column and GC-MS Solution software (v2.53). The oven temperature began at 70°C for 2 minutes, thereafter rising to 300°C at 10.0/35.0 minutes. A 4.0 µl sample was injected, using helium gas (99.995% purity) as the carrier at a flow rate of 1.5 ml/min. The injector temperature was 260°C, accompanied with a split ratio of 20. Ionization mass spectrometry used 70 eV, capturing mass spectra within the range of 40-1000 m/z for a duration of 35 minutes. Compounds were identified by juxtaposing mass spectra with the NIST Library 2008 and WILEY8 databases. Compounds were eluted, ionized, fragmented, and detected, generating mass spectrum graphs for molecular identification.

Larval/Pupal toxicity test

Laboratory colonies of mosquito larvae and pupae were used for assessing larvicidal and pupicidal activities. Twenty-five specimens of I to IV instar larvae and pupae were introduced into a 500 ml glass beaker containing 249 ml of dechlorinated water and 1 ml of the specified doses of *C. inophyllum* leaf extract. Larval sustenance was provided for the test larvae. For each concentration evaluated, 2 to 5 trials were conducted, with each trial including three duplicates. The larvae and pupae exposed to de-chlorinated water devoid of *C. inophyllum* leaf extract functioned as the control group. The control mortalities were adjusted using Abbott's formula (Abbott's, 1925).

Ovicidal Bioassay

To assess ovicidal activity, newly deposited eggs were gathered from ovitraps situated in mosquito enclosures two days subsequent to female mosquitoes consuming a blood meal. Each ovitrap included filter paper for the depositing of eggs. One hundred gravid mosquitoes were introduced into a screened enclosure containing ten oviposition cups, nine of which were filled with test solutions and one with a control (100 ml solvent, water, and Polysorbate 80). A minimum of 100 eggs were used for each treatment, with five repetitions conducted. Post-treatment, the eggs were washed, filtered, and immersed in dechlorinated water for hatching evaluation. Egg mortality was determined by non-hatchability, and hatching rates were assessed 98 hours after treatment.

Adulticidal bioassay

Adult female mosquitoes, aged 5 to 6 days and nourished with sugar, were subjected to various concentrations of *C. inophyllum* leaf extract, which was diluted in acetone and applied on filter sheets. A control sample containing ethanol was used as well. The sheets were dried overnight to facilitate the evaporation of ethanol. The bioassay was performed in two cylindrical plastic tubes measuring 125×44 mm, in accordance with WHO (1981) [12] guidelines. One tube housed the impregnated filter paper, while the other contained the mosquitoes before to and during exposure. Sucrose-fed, blood-deprived mosquitoes (20) were subjected to exposure for 1, 2, and 3 hours, with mortality documented every 10 minutes. Following exposure, mosquitoes were confined in holding tubes containing cotton pads saturated with a 10% sugar solution for a duration of 24 hours. Mortality was documented after 24 hours. The experiment was conducted in triplicate for each concentration, and fatal concentrations (LC50, LC90) were determined by probit analysis (Finney, 1971) [8].

Statistical analysis

All data were subjected to ANOVA analysis. LC50 and LC90 values, together with their 95% confidence intervals, were calculated using probit regression to examine the relationship between larval mortality and chemical concentration. The model's fit was assessed using the Chi-square test, with a p-value of less than 0.05 deemed significant. In instances of substantial divergence, a heterogeneity factor was used to calculate the 90% confidence intervals for LC_{50} and LC_{90}. Data analysis was conducted with SPSS version 16.0.

Results and Discussion

Preliminary phytochemicals analysis of *C. inophyllum* methanolic leaf extract

Bioactive compounds were identified in the methanolic leaf extract of *C. inophyllum* after phytochemical analysis. The findings are shown in Table 1.

Table 1: Preliminary phytochemicalss analysis of *C. inophyllum* leaves methanolic extract

Phytochemicals	*C. inophyllum*
Alkaloids	+
Flavonoids	++
Phenolics	++
Tannins	++
Saponin	-
Triterpenoids	-
Steroids	+
Quinones	++

- Absent, + moderate, ++ high

The methanolic leaf extract of *C. inophyllum* comprises several chemicals, indicating therapeutic potential. The results corroborate conventional uses of the herb for dermatological conditions, inflammation, and microbial infections. Following treatment with Wagner's reagent, the extract exhibited a greenish-blue hue, indicating the presence of alkaloids. This suggests nitrogenous compounds with antibacterial or antidiabetic characteristics. The methanolic leaf extract demonstrated the presence of flavonoids by turning yellow with the addition of sodium hydroxide. Flavonoids possess antibacterial, anti-inflammatory, and antioxidant properties. Upon the addition of ferric chloride, the mixture exhibited a blue-black colouration, indicating the presence of tannins. Polyphenolic tannins possess astringent, antimicrobial, and antioxidant properties. The agitation of the extract with water yielded foam, indicating the presence of saponins. Saponins has antifungal, antimicrobial, and cholesterol-lowering properties. A positive Liebermann-Burchard test indicated the presence of terpenoids, resulting in a reddish-brown colouration of the sample when treated with acetic anhydride and sulphuric acid. Terpenoids possess anti-inflammatory, anticancer, and antibacterial effects. The methanolic leaf extract lacked steroids, as shown by the absence of colour change in the acetic anhydride and sulphuric acid reagents. The methanolic leaf extract of *C. inophyllum* included alkaloids, flavonoids, tannins, saponins, and terpenoids. These components provide medicinal properties to the plant, explaining its historical use in the treatment of many ailments. These compounds have medicinal properties, facilitating the use of the plant in herbal medicine. Quantitative and in *vivo* studies are required to ascertain the pharmacological potential of these phytochemicals and investigate their therapeutic aapplications. GCMS mass spectroscopy analysis of methanolic leaf extract of *Calophyllum inophyllum* Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyse the methanolic leaf extract of *Calophyllum inophyllum*, finding its bioactive constituents (Table 2 and Fig. 1). The GC-MS analysis revealed a complex chromatogram with many peaks, indicating 20 phytocomponents, with Phytol (10.17%) and Sorbicillin (65%) as the predominant chemicals. Phytol is a diterpene alcohol with antioxidant, anti-inflammatory, anticancer, and antibacterial characteristics, rendering it significant for medicinal and pharmacological applications. It neutralises free radicals, reduces inflammation, and has potential efficacy against several cancer cell lines and microbiological infections. Sorbicillin, a polyketide molecule, has antibacterial, anticancer, antioxidant, anti-inflammatory, and immunomodulatory properties. It has shown efficacy against antibiotic-resistant microorganisms, induces apoptosis in neoplastic cells, and offers protection against oxidative stress, indicating promise in the treatment of infections, cancer, and chronic disorders. The GC-MS study underscores the plant's chemical variety and its prospective medical uses, enhancing its significance as a naturasl source of therapeutic compounds.

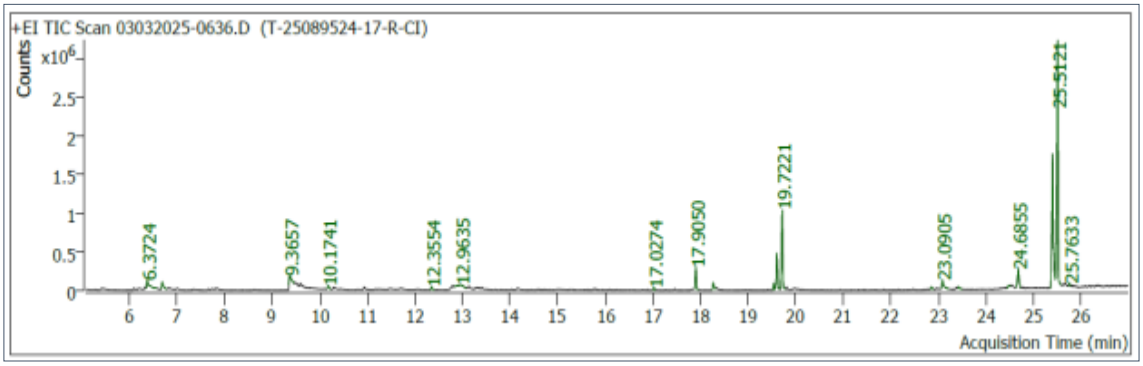

Fig 1: GCMS spectrum of methanolic leaf extract of *C. inophyllum*

Table 2: Chemical composition of *C. inophyllum* leaves methanolic extract present in GCMS analysis

S. No	Retention Time	Compound Name	Formula	Area%-T
1	6.3724	1,2-Cyclohexanedione	$C_6H_8O_2$	2.74
2	6.6892	Tetrahydrocyclopenta [1,3] dioxin-4-one	$C_7H_{10}O_3$	1.34
3	9.3657	Catechol	$C_6H_6O_2$	3.31
4	10.1741	Bis(trimethylsilyl) methylphosphonate	$C_7H_{21}O_3PSi_2$	0.62
5	12.3554	Caryophyllene	$C_{15}H_{24}$	0.4
6	12.9635	1,3-Propanediol,	$C_6H_{14}O_3$	1.78
7	17.0274	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.33
8	17.9050	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	2.9
9	18.2728	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.23
10	19.5437	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	0.84
11	19.6092	9,12,15-Octadecatrienoic acid,	$C_{19}H_{32}O_2$	5.22
12	19.7221	Phytol	$C_{20}H_{40}O$	10.17
13	22.8574	7-Hydroxyisoflavone, trimethylsilyl ether	$C_{18}H_{18}O_3Si$	0.37
14	23.0905	Hexadecanoic acid,	$C_{19}H_{38}O_4$	1.96
15	23.4328	7-Hydroxyisoflavone, trimethylsilyl ether	$C_{18}H_{18}O_3Si$	0.48
16	24.4888	9-Octadecenoic acid (Z)-,	$C_{21}H_{40}O_4$	0.71
17	24.6855	Sorbicillin, 2TMS derivative	$C_{20}H_{32}O_3Si_2$	3.18
18	25.4065	Sorbicillin, 2TMS derivative	$C_{20}H_{32}O_3Si_2$	23.89
19	25.5121	Sorbicillin, 2TMS derivative	$C_{20}H_{32}O_3Si_3$	38.18
20	25.7633	4-tert-Butyl-2-methylthiophenol,	$C_{15}H_{15}F_7OS$	0.34

Larvicidal/Pupicidal activity of methanolic leaf extract of *C. inophyllum* against dengue vector *Ae. Aegypti*

The larvicidal and pupicidal effects of methanolic leaf extract of *C. inophyllum* at various doses (100, 200, 300, 400, and 500 µg/mL) on the larval instars and pupae of the dengue vector, *Ae. aegypti*, are shown in Table 3. The death rate is 100% in I instar larvae and decreases to 95.2%, 80.8%, 74.4%, and 68.2% in II, III, IV instar larvae, and pupae, respectively, at a dose of 500 µg/mL. The determined LC_{50} values are 221.130, 245.163, 281.315, and 321.971 µg/mL for instars I to IV larvae, respectively, whereas the value for pupae has risen to 364.862 µg/mL. The derived chi-square values are 7.007, 2.080, 0.426, 0.123, and 2.352 for instars I, II, III, IV, and pupae, respectively. The calculated chi-square values indicate that there is little disparity between the predicted and actual death rates. The larvicidal and pupicidal effects demonstrated a dose-dependent escalation in mortality, with the maximum dosage (500 µg/mL) yielding the most pronounced effect on both larvae and pupae.

Table 3: Larval and pupal toxicity effect of *C. inophyllum* leaves extract on dengue vector, *Ae. Aegypti*

0	LC_{50} (LC_{90}) (µg/mL)	95% confidence Limit LC_{50} (LC_{90})	Regression equation	χ^2 (*df*=4)	
		LCL	UCL		
1st Instar	221.130 (423.890)	154.109 (358.629)	271.898 (558.478)	*y*= -1.398 +0.006 *x*	7.007 *n.s.*
2st Instar	245.163 (472.861)	221.209(437.458)	267.205(520.285)	*y* = -1.380 +0.006 *x*	2.080 *n.s.*
3st Instar	281.315 (581.559)	252.455(527.045)	308.911(660.809)	*y* = -1.201 +0.004 *x*	0.426 *n.s.*
4st Instar	321.971(660.670)	291.249(589.675)	354.549(769.539)	*y* = -1.128 +0.004 *x*	0.123 *n.s.*
Pupa	364.862(715.741)	332.700(634.022)	403.109(844.200)	*y* = -1.380 +0.004 *x*	2.352 *n.s.*

Mortality rates are means±SD of five replicates, no mortality was observed in the control Within each row, means followed by the same letter(s) are not significantly different (P<0.05), LC_{50}=lethal concentration that kills 50% of the exposed organisms, LC_{90}=lethal concentration that kills 90 % of the exposed organisms, χ^2 = *chi*-square value, *n.s.* = not significant (α=0.05)

Ovicidal activity of methanolic leaf extract of *C. inophyllum* against dengue vector *Ae. aegypti*

Table 4 presents the ovicidal activity of *Ae. aegypti* eggs after treatment with methanolic leaf extract of C. *inophyllum*. The total number of eggs used for the research was 100 for both the control and the test groups, with the control group exhibiting a hatchability rate of 98.2%. The eggs of *Ae. aegypti* subjected to several doses (100, 200, 300, 400, and 500 µg/mL) of methanolic leaf extract from *C. inophyllum* exhibited ovicidal action, leading to unsuccessful hatching. The hatchability rate was elevated at lower concentrations, but an increase in extract concentrations resulted in a reduced hatchability rate. The hatching percentage of eggs in the control medium was 98.2%, whereas the percentages for concentrations of 100, 200, 300, 400, and 500 µg/mL were 78.4%, 43.8%, and 12.6%, respectively; at 400-500 µg/mL, hatching was entirely inhibited. The findings unequivocally demonstrated that the toxicity of the methanolic leaf extract of *C. inophyllum* was contingent upon its concentration, which influences egg hatchability. The findings demonstrate that the methanolic leaf extract of *C. inophyllum* has substantial ovicidal activity, with a concentration-dependent impact seen.

Table 4: Ovicidal activity of *C. inophyllum* leaves extract against *Ae. Aegypti*

Treatment	Egg hatchability					
	Concentration (µg/mL)					
	Control	100	200	300	400	500
sC. inophyllum leaves extract	98.2±1.64[a]	78.4±1.51[b]	43.8±1.48[c]	12.6±1.14[d]	NH	NH

Values were means±SD of five replicates.
Different letters indicated significant differences (P<0.05)
NH no hatchability

Fig 2: Morphological damage analysis of *Aedes aegypti* mosquito larvae after the treatment of *C. inophyllum* extract

Conclusion

The assessment of phytochemicals and high-concentration aqueous *Calophyllum inophyllum* leaf extract in laboratory settings reveals its considerable promise as a sustainable and environmentally benign method for managing *Aedes aegypti* mosquitoes. The extract demonstrated significant larvicidal, pupicidal, ovicidal, and adulticidal effects, underscoring its broad-spectrum effectiveness across many life stages of the mosquito. The presence of bioactive phytochemicals, including flavonoids, tannins, and terpenoids, probably enhances its insecticidal characteristics. The results indicate that *C. inophyllum* leaf extract may be a feasible substitute for synthetic pesticides, especially in integrated vector management initiatives designed to diminish mosquito populations and mitigate the transmission of mosquito-borne illnesses such as dengue, Zika, and chikungunya. Additional study is advised to enhance extraction techniques, verify field effectiveness, and examine possible environmental and non-target effects to guarantee its safe and sustainable use.

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